FUNCTIONAL PROTEIN COMPOSITIONS, EMULSIONS BASED THEREON AND PROCESSES FOR THEIR PREPARATION

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Abstract of **WO9951106**

Certain lupin protein compositions demonstrate a very high degree of emulsion stabilizing functionality. This property confers the ability to stabilize emulsions at a higher ratio of oil than is possible with soya protein. Moreover, the emulsifying properties approach or even exceed those obtainable with the more expensive and often problematical animal-derived proteins such as caseinates. A process for producing such high-oil binding lupin protein compositions and emulsions has now been developed.

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TITLE

FUNCTIONAL PROTEIN COMPOSITIONS, EMULSIONS BASED THEREON AND PROCESSES FOR THEIR PREPARATION

FIELD OF THE INVENTION

The present invention relates to lupin protein compositions, and particularly to lupin protein concentrates and isolates. In particular, the invention relates to oil:water emulsions stabilized by lupin protein compositions, to gels comprising lupin protein compositions and to process for preparing highly functional lupin protein compositions and emulsions based thereon.

BACKGROUND TO THE INVENTION

Protein isolates and concentrates

The term "protein concentrate" is a term of art used to define proteinaceous preparations having a protein content of up to about 90% by weight (generally, from 50-90%, typically about 65-70%, by weight) protein. In contrast, the term "protein isolate" is generally reserved for protein preparations containing greater than about 90% (e.g., greater than about 95%) by weight protein (dry basis).

Vegetable protein concentrates and protein isolates are widely used in the food industry. Among the most popular are soya-based products, which find application for example in the manufacture of a wide variety of comminuted meat products (such as burgers and sausages) as well as in certain vegetarian products.

Protein concentrates are generally used simply as protein enrichment ingredients (nutritional adjuncts). However, protein isolates and functional concentrates also exhibit important functional properties. Among the most significant of these functional attributes is the ability to bind fat and water into stable emulsions. These emulsions may be associated with gelling, film forming, texture enhancement and stabilization in foodstuffs.

Another key functional property is the ability to form gels (especially on heating), and particularly stable gels upon cooking in the presence of water.

While it has long been recognized that the functionality of protein isolates is generally superior to that of protein concentrates, isolates are in general more expensive than concentrates. Moreover, several important aspects of the functionality of protein concentrates and isolates vary according to the nature of the protein source.

For example, and as mentioned above, the ability to emulsify high ratio oil:water mixtures at low solids concentrations is a key functional parameter, very important in for example the dairy and related industries. As used herein, the term "oil:water" is used loosely to refer to both oil-in-water and water-in-oil emulsions, though the lupin protein compositions of the invention find particular application in water-in-oil emulsions (w/o emulsions).

A high degree of functionality in this respect is exhibited by animal-derived protein concentrates and isolates (such as milk-derived proteins, e.g., whey proteins and casein(ate)), as well as by isolates from cereals (such as modified wheat glutens). Such products can stabilize emulsions in which the ratio of fat:water is 3:1, 4:1 or even greater, and such emulsions have broad utility in the dairy industry (e.g., in creams, yoghurts and ice-creams). Similar considerations apply in respect of gel forming functionality.

Unfortunately, the functionality of the widely-available and relatively inexpensive soya protein products or cereal-based proteins in respect of their emulsion-stabilizing and gel-forming activity does not match the performance attainable *via* the use of milk-based products. Moreover, the use of the latter products is associated with quite different problems: lactose intolerance and coeliac disease limits the utility of the milk and cereal products respectively, and as co-products of dairy or milling processes the base proteinaceous constituents often suffer from supply-chain difficulties (for example, associated with microbiological quality, inconsistent supply etc.).

There is therefore a need for alternative, inexpensive, broadly acceptable and effective functional protein concentrates and isolates.

Lupins and lupin proteins

Lupins have long been recognized as a viable alternative to soya as a source of vegetable protein for human consumption. The lupin plant thrives on agronomically less desirable areas and produces good yields on inferior soil types relative to other legumes. *Lupinus albus*, the white lupin, is the preferred species for cultivation in Europe, while *L. angustifolius* is the species of choice in the less fertile soil of Australia.

It has long been known that the protein content of lupin seeds is equal to that of whole soya beans, and it has been exploited for years as a source of (non-functional) protein in animal feeds.

Moreover, lupin concentrates and isolates *per se* are known (see e.g., WO97/12524 and EP0522800, referred to *infra*), and these isolates/concentrates are also known to affect the chemical/physical behaviour of foodstuffs in which they are incorporated (*ibidem*).

However, the present inventors have now unexpectedly discovered that a very high degree of emulsion stabilizing functionality may be associated with lupin protein compositions. This extremely useful property has hitherto gone unrecognized. It confers the ability to stabilize emulsions at a higher ratio of oil than is possible with soya protein, and indeed the emulsifying properties approach (or even exceed) those obtainable with the (more expensive and often problematical) animal-derived proteins (such as caseinates).

Strikingly, it has been found that even at relatively low levels of purity (e.g., 20-40%), lupin protein compositions provide an unusually high degree of oil binding capability whilst having higher levels of fibre.

Furthermore, it has also now been found that post-isoelectric precipitation and/or washing of the lupin protein according to the methods taught in our earlier EP0522800 (discussed *infra*) or restructuring of lupin protein compositions howsoever prepared yield products (e.g., isolates and concentrates) having a stabilizing activity which may equal (or even exceed) that of caseinate (particularly when the lupin protein is present at relatively high levels of purity, for example as a lupin protein composition at greater than 70% purity).

Lupin protein products which have been held at an alkaline pH at an elevated temperature and then neutralized (for example, by application of the relevant process steps described in EP0522800) are referred to herein as "restructured" lupin proteins. Without wishing to be bound by any theory, it is thought that the restructuring treatment promotes conformational changes in some or all of the lupin proteins such that the surface charge profiles (and hence the fat/water binding characteristics) of the proteins are modified and the oil-binding functionality dramatically improved.

SUMMARY OF THE INVENTION

According to a first aspect, the present invention there is provided a process for producing a high-oil binding lupin protein composition comprising the steps of holding an aqueous slurry of a lupin protein composition at an alkaline pH at an elevated temperature and neutralising the treated slurry to produce a composition comprising restructured lupin protein.

Preferably, the process is for producing a high-oil lupin protein stabilized emulsion, the process further comprising the step of mixing the restructured lupin protein with oil and water to form a high-oil lupin-based emulsion, and the lupin protein composition used as the starting material may be least partially denatured.

In a second aspect, the invention provides a process for producing a high-oil lupin protein stabilized emulsion comprising the steps of holding an aqueous slurry of a lupin protein composition at an alkaline pH at an elevated temperature, neutralising the treated slurry to produce a composition comprising restructured lupin protein (and optionally further comprising the step of evaporating and drying (e.g., by spray drying) the neutralised slurry) and mixing the restructured lupin protein with oil and water to form a high-oil lupin-based emulsion.

The restructured lupin protein preferably comprises high-oil binding lupin protein and the lupin protein composition used as the starting material may be at least partially denatured.

In a third aspect, the invention provides a process for producing a lupin protein composition comprising the steps of providing a composition comprising at least partially denatured lupin protein, holding an aqueous slurry of the at least partially denatured lupin protein composition at an alkaline pH at an elevated temperature and neutralising the treated slurry to produce a composition comprising restructured lupin protein.

Preferably, the restructured lupin protein comprises high-oil binding lupin protein. The process finds particular application in the production of a high-oil lupin protein stabilized emulsion, when the process further comprising the step of mixing the restructured lupin protein with oil and water to form a high-oil lupin-based emulsion.

The holding step may be performed on an isoelectrically precipitated lupin protein composition, though any treatment which removes oligosaccharides and/or colours and/or flavours from the lupin protein composition may be used. Preferred is alcohol washing and/or ultrafiltration.

Such removal steps may be performed prior to the holding step or after the neutralizing step.

As used herein, the term "high-oil binding lupin protein composition" may define compositions in which the lupin proteins are present in a form such that their oil-binding functionality is enhanced relative to native lupin protein compositions. Preferably, the oil-binding functionality of such high-oil binding compositions is enhanced to an extent such that the compositions are caseinate mimetic. The term "caseinate mimetic" is used herein to defines an emulsifying functionality broadly mimetic of caseinate, and in particular an oil-binding functionality broadly mimetic of caseinate. Thus, caseinate mimetics may functionally replace caseinate in a wide range of different foodstuffs and functional food ingredients, and may therefor constitute caseinate replacers.

The term "high-oil binding lupin protein composition" is also used herein to refer to compositions which are capable of forming a firm emulsion. The term "firm emulsion" is a term of art defining solid, semi-solid, gel-like or highly viscous oil:water emulsions (either oil-in-water or water-in-oil). Such emulsions may be cheese-like or cream-like in texture.

The "high-oil binding lupin compositions" may have a fat binding functionality which exceeds that of caseinate. The term is therefore also used herein to define compositions which are capable of forming an emulsion comprising fat and water in the ratio 3:1 or greater (e.g., 4:1 or greater); and/or capable of forming an emulsion at a protein composition:water:fat ratio (dry weight basis) of 1:3:12, 1:4:16, 1:5:20, 1:6:24, 1:7:28, 1:8:32, 1:9:36 or 1:10:40; and/or capable of forming an emulsion comprising 1 part by weight protein composition (dry basis) and: (i) at least 5 parts by weight water and at least 5 parts by weight fat; or (ii) at least 10 parts by weight water and at least 20 parts by weight fat; or (iii) at least 15 parts by weight water and at least 30 parts by weight fat; or (iv) at least 20 parts by weight water and at least 40 parts by weight fat; or (v) at least 2, 5, 10, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 or >1000 parts by weight water plus oil (combined).

Preferably, the lupin protein compositions are capable of forming an emulsion wherein the ratio of parts by weight fat:water in the emulsion is: (i) greater than 1:1; or (ii) greater than 3:2; or (iii) greater than 2:1; or (iv) greater than 5:2; or (v) greater than 3:1;

or (vi) greater than 7:2; or (vii) greater than 4:1; or (viii) varies between 2:1 to 1000:1 or >1000:1; or (ix) selected from any or the ratios: 5:1, 10:1, 100:1, 200:1, 300:1, 400:1, 500:1, 600:1, 700:1, 800:1, 900:1, 1000:1 or >1000:1. Such compositions are preferably capable of stabilizing emulsions at levels of one part by weight (relative to the weight of the fat and water components) lupin protein composition, e.g., to yield stable emulsions having a protein composition:water:fat of 1:100:800.

Particularly preferred are "high-oil binding lupin compositions" which are capable of forming an emulsion comprising fat and water in the ratio protein composition:water:fat of (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 or 1.0):2:8, or (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 or 1.0):5:15 or (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 or 1.0):5:30 or (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 or 1.0):5:40.

In preferred embodiments, the aqueous slurry to be treated has a solids content of 12-25% by weight, e.g., 12-17% by weight. For some applications (e.g., creams), the solids content may be lower (e.g., 2-5%).

The alkaline pH is 7.5-9.5, for example 7.5-9.5 (e.g., about 8.5) and/or the neutralising pH is 6.5-7.5, while the elevated temperature may be any of: (a) 25-35, e.g., about 30, (b) 35-45, e.g., about 40, (c) 45-55, (d) 55-65, (e) 65-75, (f) 75-85, (g) 80-95, e.g., about 85°C.

The solubility (or PDI) of the lupin protein is preferably decreased and the oil-binding capacity of the protein may be increased by the treatment.

The holding and neutralising steps are preferably carried out in the absence of substantial shearing forces.

The process of the invention preferably produce lupin protein compositions or restructured lupin protein compositions having:

(a) at least 20% (dry weight basis) lupin protein;
(b) at least 30% (dry weight basis) lupin protein;
(c) at least 40% (dry weight basis) lupin protein;
(d) at least 60% (dry weight basis) lupin protein;
(e) at least 70% (dry weight basis) lupin protein;
(f) at least 90% (dry weight basis) lupin protein;
(g) at least 95% (dry weight basis) lupin protein;
(h) greater than 95% (dry weight basis) lupin protein;
(i) a lupin protein concentrate;
(j) a lupin protein isolate.

The lupin protein compositions or restructured lupin protein compositions may therefore be lupin protein concentrates or isolates.

The holding step may be for 1-180 minutes (for example 1-120, e.g., 1-60 minutes). Shorter holding times may result in products which form stable emulsions having a softer or creamier texture.

The processes of the invention preferably further comprise the step of evaporating and drying (e.g., by spray drying) the lupin protein composition product, restructured lupin protein composition or neutralised slurry.

In preferred embodiments where non-defatted lupin protein grits are used, the lupin protein composition is:

(a) treated to remove rancidity-promoting activity (for example, enzyme (e.g. lipase) activity); and/or

(b) treated to substantially eliminate enzyme (e.g. lipase) activity; and/or

(c) derived from a blanched lupin source (e.g. blanched lupin grits); and/or

(d) enzyme-inactivated lupin meal,

for example wherein the lupin protein is at least partially inactivated (e.g., as a result of the treatment, blanching or enzyme inactivation).

Also contemplated by the invention is a lupin protein composition or emulsion obtainable by the processes of the invention.

According to a fourth aspect, the present invention provides an emulsion comprising a lupin protein composition, water and fat, wherein the lupin protein is present in an amount sufficient to stabilize the emulsion.

The term "fat" is used herein to include fats which are liquid at room temperatures (often referred to as oils).

The emulsion may contain any suitable ratio of protein composition:(water plus oil). Typically, the food industry requires emulsions of weight ratio 1:3:6, 1:2:8 or 1:5:5 (protein composition (e.g., isolate or concentrate):water:fat), particularly in stabilizing food systems that use high fat and water mixtures, such as comminuted meat products (e.g., sausages) and dairy product replacers. With existing materials, this can generally be achieved by using protein isolates but not by using protein concentrates.

For most food applications, however, it is desirable to keep the solid protein content low relative to the fat and water. Thus, the emulsion preferably comprises 1 part by weight protein composition (dry basis) and: (a) at least 5 parts by weight water and at least 5 parts by weight fat; or (b) at least 10 parts by weight water and at least 20 parts by weight fat; or (c) at least 15 parts by weight water and at least 30 parts by weight fat; or (d) at least 20 parts by weight water and at least 40 parts by weight fat.

Alternatively, the emulsion may comprise 1 part by weight protein composition (dry basis) and: (a) at least 2, 5, 10, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 or >1000 parts by weight water plus oil (combined).

The ratio of fat to water in the emulsion may vary over a broad range, and may be selected *inter alia* according to the ultimate use to which the emulsion is to be put. In general, high oil:water ratios are desirable (particularly at low solids content - see above), and so preferably the ratio of oil:water is: (a) greater than 1:1; or (b) greater than 3:2; or (c) greater than 2:1; or (d) greater than 5:2; or (e) greater than 3:1.

However, the lupin proteins of the invention have been found to effectively stabilize emulsions containing very high oil:water ratios, for example emulsions wherein the oil:water ratio varies between 2:1 to 1000:1 (or even higher). Thus, particularly preferred are emulsions wherein the ratio of oil:water is selected from any of the ratios: 5:1, 10:1, 100:1, 200:1, 300:1, 400:1, 500:1, 600:1, 700:1, 800:1, 900:1, 1000:1 or >1000:1. In such emulsions, the protein composition may be present at one part per weight relative to the weight of the water and oil.

Since it is envisaged that the lupin-based emulsions of the present invention will provide an effective alternative to caseinate, the emulsion of the invention may be formulated such that its fat:water ratio is mimetic of a caseinate-stabilized emulsion.

The lupin protein content of the lupin protein composition component of the emulsion may vary over a broad range. Those, skilled in the art will be able to select the appropriate concentration on the basis of the ultimate application, the physical state of the lupin protein (i.e., whether native, more or less denatured, derivatized, sub-fractionated etc.) and the nature of the oil component.

It will be appreciated that functionality at any given concentration can be readily determined using routine tests.

For most applications, the lupin protein composition will comprise: (a) at least 20% lupin protein; or (b) at least 25% lupin protein; or (c) at least 30% lupin protein; or (d) at least 35% lupin protein; or (e) at least 40% lupin protein; or (f) at least 45% lupin protein; or (g) at least 50% lupin protein; or (h) at least 55% lupin protein; or (i) at least 60% lupin protein; or (j) at least 65% lupin protein; or (k) at least 70% lupin protein; or (l) at least 75% lupin protein; or (m) at least 80% lupin protein; or (n) at least 85% lupin protein; or (o) at least 90% lupin protein; or (p) at least 95% lupin protein.

Particularly preferred for use in the emulsions of the invention are lupin protein concentrates or lupin protein isolates (as hereinbefore defined).

As described in more detail below, the lupin protein for use according to the invention may be provided in any suitable form or physical state. Preferably, it is present in partially denatured form, since this is usually associated with higher functionality. It is also preferably debittered, for the reasons described in more detail below.

The lupin protein for use in the invention is preferably prepared by isoelectric precipitation and/or washing (for example as described below).

Also contemplated by the invention is the emulsion as described above in gelled form (a form which may arise after cooking in a foodstuff).

In another aspect, the invention relates to a gel comprising a lupin protein composition (as hereinbefore defined) and water.

Also covered by the invention are various functional food ingredients comprising the emulsion or gel of the invention. Here, the invention also finds utility as a functional ingredient in various foodstuffs, drinks (e.g., energy or sports drinks) and animal feeds.

For example, the emulsion and/or gel may be used as an ingredient in a baby food, bakery product (for example, a bread, yeasted good or cake) or bakery supply product (for example, a custard or a bakery filling or topping), a batter or breading, cereal, confectionary, flavour or beverage emulsion, fruit filling, gravy, soup, sauce or food thickener, frozen, chilled or ambient stable gravy, soup, sauce or food thickener, pasteurized, retorted or UHT treated gravy, soup, sauce or food thickener, meal or meal component, e.g., a vegetarian meal/component, meat product (e.g., a comminuted meat product, sausage, burger, grillsteak, canned meat, meat pie, fish, meat spread and paste), petfood, pharmaceutical or neutraceutical, potato product, dairy product (e.g., an ice-cream, dessert, milk drink, milk shake, yoghurt, cheese, cheese spread or dip), dressing (e.g. ,a salad or low fat dressing), snack or cracker, spread (e.g., savoury or sweet spread), pasta product (e.g., a noodle), fat-filled powder, quiche or flan, textured vegetable protein, vegetarian grillsteak, pate (e.g., vegetarian pate) or spread, vegetable or meat extract, low fat spread, cheese or cream mimetic.

The invention also contemplates cosmetics, for example a cream (e.g., face cream), lipstick, deodorant carrier, lotion, hair gel, soap (e.g., liquid soap) or skin care product (e.g., sun lotion).

In another aspect, the invention relates to a process for the production of an emulsion as hereinbefore defined comprising the steps of: (a) providing a lupin protein composition; (b) mixing the protein composition of step (a) with oil and water; (c) emulsifying the mixture of step (b).

Preferably, the process of the invention is carried out *in situ* within a foodstuff, drink or animal feed during processing thereof. However, it may also be carried out outside a foodstuff (e.g., in vitro) in order to produce an emulsion useful *per se* as a functional food ingredient.

In yet another aspect, the invention relates to a process for the production of a gel as hereinbefore defined comprising the steps of: (a) providing a lupin protein composition; (b) mixing the protein composition of step (a) with water; (c) gelling the mixture of step (b), for example by heating.

In the processes of the invention, step (a) may comprise the step of isoelectrically precipitating lupin protein.

In particularly preferred embodiments, step (a) further comprises the preliminary steps of: (a) providing lupin seeds; and (b) debittering the lupin seeds (e.g., by a two stage extraction as described in WO97/12524 (PCT/DE 96/01915).

Preferably, the process of the invention further comprises "restructuring" isoelectrically-prepared lupin protein by subjecting the protein to a holding step at alkaline pH at an elevated temperature followed by neutralisation. Preferably, this process is applied to partially denatured lupin protein. It may also be applied to isoelectrically precipitated lupin protein (which may or may not be partially denatured). Thus, the process may involve the post-isoelectric precipitation and/or washing steps of, in the absence of substantial shearing forces: (a) holding an aqueous slurry of the isolectrically-prepared protein at an alkaline pH and a treatment temperature of 75-95°C for 1-120 minutes (e.g., 1 to 60 min); (b) neutralizing the treated slurry e.g., to a pH of 6.8 to 7.0; and optionally (c) evaporating the neutralized slurry and drying it (for example by spray drying).

In this embodiment (and when the product is to be spray dried), the aqueous slurry preferably has a solids content of 12-25% by weight, e.g., 12-17% by weight. If the product is to be dried by other processes (such as filter pressing and/or alcohol precipitation), the solids content can be higher.

The alkaline pH is preferably 7.5-9.0 or 9.5, e.g., 7.5-8.5, while the treatment temperature may be 80-95°C, for example about 85°C. In such processes, the structural integrity and/or solubility of the lupin protein may be decreased (at least to some extent) by the treatment (without necessarily compromising, and in some circumstances actually improving, functionality). Thus, the treatment may effect a degree of denaturation of the native lupin proteins (as discussed in our earlier EP0522800).

In another aspect, the invention relates to a method for increasing the oil binding functionality of a lupin protein composition comprising the steps of:

(a) holding an aqueous slurry of a lupin protein composition at an alkaline pH at an elevated temperature; and

(b) neutralising the treated slurry to produce a composition comprising restructured lupin protein (and optionally further comprising the step of evaporating and drying (e.g. by spray drying) the neutralised slurry).

In another aspect, the invention provides a method for increasing the oil binding functionality of a lupin protein composition comprising the steps of:

(a) providing a composition comprising at least partially denatured lupin protein;

(b) holding an aqueous slurry of the at least partially denatured lupin protein composition at an alkaline pH at an elevated temperature; and

(c) neutralising the treated slurry to produce a composition comprising restructured lupin protein (and optionally further comprising the step of evaporating and drying (e.g., by spray drying) the neutralised slurry).

All of the preferred features of the invention as discussed above may also feature *mutatis mutandis* in these methods of the invention.

DETAILED DESCRIPTION

Lupin proteins for use in the invention

The lupin proteins for use in the invention may be any protein extracted or derived from a member of the genus *Lupinus*. For food applications, preferred are *L. albus* and *L. angustifolius*. For other applications (such as lubricants, paints and fillers), bitter lupins can be used.

The lupin proteins need not be purified to homogeneity, either with respect to other species of lupin protein present or with respect to other (non-proteinaceous) components. Indeed, for most applications the lupin protein composition will comprise a heterogeneous mixture of different lupin proteins, including storage proteins, enzymes, structural proteins (including membrane proteins, fibrous proteins and globular proteins), together with contaminating carbohydrate, cellulosic and fatty materials (often in minor amounts).

For some applications, it may be advantageous to subfractionate the lupin protein fraction (for example, to enrich for globulin storage proteins), for example in order to optimize the structural characteristics, solubility, charge or amino acid profile of the lupin protein. It may also be desirable to derivitize or physically modify the lupin protein, for example by (at least partially) denaturing the proteins (e.g., by heating) or by (e.g., partial) enzymic digestion (e.g., protease treatment to yield peptides).

Any of the above approaches can be used to modify the fat/water binding characteristics of the lupin protein and so optimise the emulsion stabilizing properties for any given application.

The lupin proteins may be extracted by any of a variety of standard protein extraction techniques (including those commonly employed in soya bean processing). For most applications, the lupin plants and/or seeds are comminuted (e.g., by milling or grinding) before the extraction processes to enlarge the surface area and maximize yield.

Where lupin seeds form part of the starting material, the seeds are preferably pre-processed. This may involve shelling, washing and/or sieving.

Preferably, the lupin proteins are extracted by processes which do not substantially denature the lupin proteins, although lupin protein compositions containing significant amounts of denatured proteins may be useful (or even advantageous) in some applications, as described in more detail below.

More highly functional lupin compositions may be produced by rendering the proteins insoluble by acid washing at the isoelectric point, so that unwanted carbohydrates

are washed away. The less acidic whey fraction containing active enzymes is also removed at this stage as a relatively minor protein subfraction.

Compositions produced by such processes contain residual, high molecular weight, insoluble carbohydrates of arabinogalactan composition, concentrated by the process to at least 25% by weight, often at least 40% by weight.

Highly functional protein isolates may be produced for example by acid washing at the isoelectric point, followed by dissolving the washed slurry in alkali, separating the solid residue containing insoluble carbohydrates from the solution and re-precipitating the protein at e.g., pH 7.0. The products of such processes are referred to herein as isoelectrically-prepared protein products, and the process is referred to as isoelectric precipitation/washing.

Protein of even higher functionality may be obtained by further downstream processing after precipitation/washing, for example as described *infra* and in our earlier EP0522800. (The disclosure of the post-isoelectric precipitation/washing steps as defined in the description and claims of this document are incorporated herein by reference.)

In particular, it has now surprisingly been found that the functionality of lupin proteins which have been isoelectrically prepared can be enhanced by the steps of, in the absence of substantial shearing forces: (a) holding an aqueous slurry of the isolectrically-prepared protein at an alkaline pH and a treatment temperature of 75-95°C for 1-120 minutes (e.g., 1 to 60 min); (b) neutralizing the treated slurry e.g., to a pH of 6.8 to 7.0; and optionally (c) evaporating the neutralized slurry and drying it (for example by spray drying).

The aqueous slurry may have a solids content of 12-25% by weight, e.g., 12-17% by weight. The alkaline pH may be 7.5-9.0 or 9.5, e.g., 7.5-8.5. The treatment temperature may be 80-95°C, for example about 85°C. The solubility of the lupin protein may be somewhat decreased by the treatment.

However, it has also surprisingly been found that *untreated* lupin protein compositions (even containing low concentrations, such as 35-40% lupin protein on a dry weight basis) provide an unusually high degree of oil binding activity.

Rancidity

Many lupin protein preparations develop rancid odours and/or flavours on storage. Such problems may arise from endogenous enzyme (e.g., lipase and/or lipoxygenase) activity. Thus, the lupin starting materials and/or products of the invention are preferably treated so as to remove or inactivate factors which promote rancidity.

Thus, rancidity-promoting enzyme activities are preferably inactivated prior to further processing (or as a final processing step). Such inactivation may be achieved for example by blanching (e.g., by steaming), though any other suitable technique may be employed. The treatments may effect denaturation of one or more enzyme activities (e.g., lipase and/or lipoxygenase) in the lupin material, and particularly preferred is a blanching treatment.

The process parameters of the blanching treatment are selected so as to substantially destroy endogenous lipase and/or esterase activity (as measured e.g., by the indoxyl acetate test described in Purr (1990) Zucker-und-Suesswarenwirtschaft, 43 (1) 20-22. The appropriate temperature, pressure and time parameters can be readily determined by routine trial and error by those skilled in the art. Preferred are temperatures in the range 60-130°C, and particularly preferred are temperatures of at least 90°C (e.g., between 90-100°C). For example, the material may be held for about 5 min at 95°C, or autoclaved at a temperature of 100°C or above.

Such treatments may at least partially denature the bulk lupin proteins. However, it has surprisingly been found that when such partially denatured lupin proteins are subjected to the restructuring process described *infra*, products having greater oil binding functionality relative to restructured native protein may be obtained.

Lupin anti-nutrient removal

Lupin protein often contains so-called anti-nutrients. These constituents may be toxic, unpalatable or induce undesirable consequences during digestion (such as flatulence). They include bitter constituents (such as alkaloids) and certain sugars (including oligosaccharides).

The lupin proteins for use in the invention are preferably free from these anti-nutrients, and so the proteins are preferably processed such that anti-nutrients are removed.

Many different processes are available for removing the anti-nutrients (often referred to in the art as "debittering treatments"), and those skilled in the art will readily be able to effect a suitable protocol. For example, the lupin protein may be treated by the two stage extraction processes disclosed in WO97/12524 (PCT/DE96/01915) (the teaching of which is incorporated herein by reference).

EXAMPLES

EXAMPLE 1

POULTRY SKIN EMULSION

The high fat binding characteristics of lupin protein make it ideally suited to the production of poultry skin emulsions. Typically, poultry skin emulsions are used as a cheap filler within reformed poultry products such as poultry burgers.

4 kg of poultry skin are chopped in a bowlcutter at high speed for 15 seconds. The cutter speed is then set to slow speed and 0.5 kg of a lupin protein concentrate (ca. 70% lupin protein) and 2.0 kg of water are added. The chopping is continued for two minutes on high speed until a smooth glossy emulsion has been formed. The emulsion is then chilled and used as an ingredient in a poultry burger.

EXAMPLE 2

FRANKFURTER

1.75 kg pork belly 50 vl, 1.5 kg pork shoulder 80 vl and 0.5 kg of beef flank 70 vl are ground in a mincer with a 10 mm plate. The ground meats are then placed in a mixer together with 0.05 kg of lupin protein concentrate (ca. 70% lupin protein), 0.9 kg water, 0.1 kg curing salt and 0.1 kg seasoning, and mixed for two minutes.

0.1 kg of potato starch is then added and mixing continued for a further minute. The mix is then passed through an emulsion mill with a 0.5 mm plate and filled into casings. The products are steam cooked at 80°C to an internal temperature of 72°C, then cooled and peeled.

EXAMPLE 3

NON-DAIRY SPREAD

7 kg of rape, soya and palm fat blend are heated to 45°C and 0.1 kg of dairy flavour mixed in. 0.8 kg of lupin isolate (ca. 90% lupin protein) is added to 2.1 kg of water and heated to 80°C. This liquid phase is then slowly added to the oil phase using a Y-Tron homogenizer. The mixture is then poured into containers and chilled.

EXAMPLE 4

PREPARATION OF RESTRUCTURED LUPIN PROTEIN COMPOSITION (40% LUPIN PROTEIN)

Lupin meal was steamed for 5 min at 95°C and tested to confirm that endogenous lipase activity had been eradicated. The blanched meal was dispersed to 17% solids in water and adjusted to pH 4.5 using hydrochloric acid. The dispersion was passed through a centrifugal separator to remove oligosaccharides. The resulting slurry was made to 14% solids, adjusted to pH 8.5 by the addition of sodium hydroxide and passed through a scraped-surface heat exchanger to raise the temperature to 90°C. The slurry was then neutralized by the addition of hydrochloric acid and spray dried.

EXAMPLE 5

ICE-CREAM

A full-fat ice-cream was prepared according to the following recipe:

water	47.5%
double cream	25.8%
sugar	12.0%
skimmed milk powder	10.0%
dextrose	4.0%
emulsifier & stabilizer blend	0.5%
flavouring/colouring	0.2%

The lupin protein composition prepared as described in Example 4 could functionally replace the skimmed milk powder and the emulsifier/stabilizer blend in the above recipe with no loss of quality.

CLAIMS:

1. A process for producing a high-oil binding lupin protein composition comprising the steps of:
   (a) holding an aqueous slurry of a lupin protein composition at an alkaline pH at an elevated temperature; and optionally
   (b) neutralising the treated slurry to produce a composition comprising restructured lupin protein.

2. A process for producing a high-oil lupin protein stabilized emulsion comprising the steps of:
   (a) holding an aqueous slurry of a lupin protein composition at an alkaline pH at an elevated temperature;
   (b) mixing the restructured lupin protein with oil and water to form a high-oil lupin-based emulsion,

wherein the treated slurry of step (a) is for example neutralised to produce a composition comprising restructured lupin protein (optionally further comprising the step of evaporating and drying (e.g., by spray drying) the neutralised slurry).

3. A process for producing a lupin protein composition comprising the steps of:
   (a) providing a composition comprising at least partially denatured lupin protein;
   (b) holding an aqueous slurry of the at least partially denatured lupin protein composition at an alkaline pH at an elevated temperature; and optionally
   (c) neutralising the treated slurry to produce a composition comprising restructured lupin protein.

4. The process of Claim 1 wherein:
   (a) the process is for producing a high-oil lupin protein stabilized emulsion, the process further comprising the step of mixing the restructured lupin protein with oil and water to form a high-oil lupin-based emulsion; and/or
   (b) the lupin protein composition of step (a) is at least partially denatured.

5. The process of Claim 2 wherein:
   (a) the restructured lupin protein comprises high-oil binding lupin protein; and/or
   (b) the lupin protein composition of step (a) is at least partially denatured.

6. The process of Claim 3 wherein:
   (a) the restructured lupin protein comprises high-oil binding lupin protein; and/or
   (b) the process is for producing a high-oil lupin protein stabilized emulsion, the process further comprising the step of mixing the restructured lupin protein with oil and water to form a high-oil lupin-based emulsion.

7. The process of any one of the preceding claims wherein the holding step is performed on an isoelectrically precipitated lupin protein composition.

8. The process of any one of Claims 1-6 further comprising the step of removing oligosaccharides and/or colours and/or flavours from the lupin protein composition, for example by:

(a) isoelectric precipitation/washing; and/or

(b) alcohol washing; and/or

(c) ultrafiltration.

9. The process of Claim 8 wherein the removal step is performed prior to the holding step or after the neutralizing step.

10. The process of any one of Claims 1, 5, 6 and claims dependent thereon wherein the high-oil binding lupin protein composition:

(a) is a caseinate mimetic; and/or

(b) is capable of forming a firm emulsion; and/or

(c) is capable of forming an emulsion comprising fat and water in the ratio 3:1 or greater (e.g., 4:1 or greater); and/or

(d) is capable of forming an emulsion at a protein composition:water:fat ratio (dry weight basis) of 1:3:12, 1:4:16, 1:5:20, 1:6:24, 1:7:28, 1:8:32, 1:9:36 or 1:10:40; and/or

(e) is capable of forming an emulsion comprising 1 part by weight protein composition (dry basis) and:

(i) at least 5 parts by weight water and at least 5 parts by weight fat; or

(ii) at least 10 parts by weight water and at least 20 parts by weight fat; or

(iii) at least 15 parts by weight water and at least 30 parts by weight fat; or

(iv) at least 20 parts by weight water and at least 40 parts by weight fat; or

(v) at least 2, 5, 10, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 or >1000 parts by weight water plus oil (combined); and/or

(f) is capable of forming an emulsion wherein the ratio of fat:water in the emulsion is:

(i) greater than 1:1; or

(ii) greater than 3:2; or

(iii) greater than 2:1; or

(iv) greater than 5:2; or

(v) greater than 3:1; or

(vi) greater than 7:2; or

(vii) greater than 4:1; or

(viii) varies between 2:1 to 1000:1 or >1000:1; or

(ix) selected from any or the ratios: 5:1, 10:1, 100:1, 200:1, 300:1, 400:1, 500:1, 600:1, 700:1, 800:1, 900:1, 1000:1 or >1000:1; and/or

(g) exceeds the oil binding functionality of a soya protein isolate on a weight for weight basis.

11. The process of Claim 10(b) wherein the firm emulsion is solid or semi-solid (e.g., being cheese-like or cream-like in texture).

12. The process of any one of the preceding claims wherein the aqueous slurry to be treated has a solids content of 12-25% by weight, e.g., 12-17% by weight.

13. The process of any one of the preceding claims wherein the alkaline pH is 7.5-9.5, for example 7.5-9.5 (e.g., about 8.5) and/or the neutralising pH is 6.5-7.5.

14. The process of any one of the preceding claims wherein the elevated temperature is any of:

(a) 25-35, e.g., about 30°C

(b) 35-45, e.g., about 40°C

(c) 45-55, e.g., about 50°C

(d) 55-65, e.g., about 60°C

(e) 65-75, e.g., about 70°C

(f) 75-85, e.g., about 80°C

(g) 80-95, e.g., about 85°C.

15. A process according to any one of the preceding claims wherein the solubility of the protein is decreased and the oil-binding capacity of the protein is increased by the treatment.

16. The process of any one of the preceding claims wherein the holding and neutralising steps are carried out in the absence of substantial shearing forces.

17. The process of any one of Claims 1-16 wherein the lupin protein composition product or restructured lupin protein composition is:

(a) at least 20% (dry weight basis) lupin protein;

(b) at least 30% (dry weight basis) lupin protein;

(c) at least 40% (dry weight basis) lupin protein;

(d) at least 60% (dry weight basis) lupin protein;

(e) at least 70% (dry weight basis) lupin protein;

(f) at least 90% (dry weight basis) lupin protein;

(g) at least 95% (dry weight basis) lupin protein;

(h) greater than 95% (dry weight basis) lupin protein;

(i) a lupin protein concentrate;

(j) a lupin protein isolate.

18. The process of any one of the preceding claims wherein the holding step is for 1-180 minutes (for example 1-120, e.g., 1-60 minutes).

19. The process of any one of Claims 1, 3 and claims dependent thereon further comprising the step of evaporating and drying (e.g. by spray drying) the lupin protein composition product, restructured lupin protein composition or neutralised slurry.

20. The process of any one of the preceding claims wherein the lupin protein composition in step (a) is:

(a) treated to remove rancidity-promoting activity (for example, enzyme (e.g., lipase) activity); and/or

(b) treated to substantially eliminate enzyme (e.g., lipase) activity; and/or

(c) derived from a blanched lupin source (e.g., blanched lupin grits); and/or

(d) enzyme-inactivated lupin meal,

for example wherein the lupin protein is at least partially inactivated (e.g. as a result of the treatment, blanching or enzyme inactivation).

21. The emulsion of any one of Claim 2 and claims dependent thereon wherein the emulsion has the characteristics defined in Claim 10(b), (c), (d) or (f).

22. A lupin protein composition or emulsion obtainable by the process of any one of the preceding claims.

23. An emulsion (e.g., as defined in Claim 10(b), (c), (d) or (f) or as claimed in claim 22) comprising:

(a) a lupin protein composition (e.g., as claimed in Claim 22);

(b) water; and

(c) fat,

the lupin protein being present in an amount sufficient to stabilize the emulsion.

24. The emulsion of Claim 23 comprising 1 part by weight protein composition (dry basis) and:

(a) at least 5 parts by weight water and at least 5 parts by weight fat; or

(b) at least 10 parts by weight water and at least 20 parts by weight fat; or

(c) at least 15 parts by weight water and at least 30 parts by weight fat; or

(d) at least 20 parts by weight water and at least 40 parts by weight fat; or

(e) at least 2, 5, 10, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 or >1000 parts by weight water plus oil (combined).

25. The emulsion as defined in any one of the preceding claims wherein the ratio of fat:water in the emulsion is:

(a) greater than 1:1; or

(b) greater than 3:2; or

(c) greater than 2:1; or

(d) greater than 5:2; or

(e) greater than 3:1; or

(f) greater than 7:2; or

(g) greater than 4:1; or

(h) varies between 2:1 to 1000:1 or >1000:1; or

(i) selected from any or the ratios: 5:1, 10:1, 100:1, 200:1, 300:1, 400:1, 500:1, 600:1, 700:1, 800:1, 900:1, 1000:1 or >1000:1.

26. The emulsion as defined in any one of the preceding claims having a fat:water ratio mimetic of a milk protein- (e.g., caseinate-) stabilized emulsion.

27. The emulsion as defined in any one of the preceding claims wherein the protein composition comprises:

(a) at least 20% lupin protein; or

(b) at least 25% lupin protein; or

(c) at least 30% lupin protein; or

(d) at least 35% lupin protein; or

(e) at least 40% lupin protein; or

(f) at least 45% lupin protein; or

(g) at least 50% lupin protein; or

(h) at least 55% lupin protein; or

(i) at least 60% lupin protein; or

(j) at least 65% lupin protein; or

(k) at least 70% lupin protein; or

(l) at least 75% lupin protein; or

(m) at least 80% lupin protein; or

(n) at least 85% lupin protein; or

(o) at least 90% lupin protein; or

(p) at least 95% lupin protein.

28. The emulsion as defined in any one of the preceding claims wherein the protein composition is a lupin protein concentrate or isolate.

29. The emulsion as defined in any one of the preceding claims wherein the lupin protein is:

(a) in substantially native form; and/or

(b) is debittered.

30. The emulsion as defined in any one of the preceding claims wherein the lupin protein is an isoelectrically precipitated lupin protein.

31. The emulsion of Claim 30 wherein the lupin protein comprises a restructured lupin protein.

32. The emulsion as defined in any one of the preceding claims in gelled form.

33. A gel comprising a lupin protein or lupin protein composition as defined in any one of the preceding claims and water.

34. A functional food ingredient comprising the emulsion or gel as defined in any one of the preceding claims.

35. A foodstuff, drink (e.g., energy or sports drink) or animal feed comprising the emulsion or gel of any one of Claims 23 to 34.

36. The foodstuff of Claim 35 which comprises:

(a) a babyfood; or

(b) a bakery product (for example, a bread, yeasted good or cake) or a bakery supply product (for example, a custard or a bakery filling or topping); or

(c) a batter or breading;

(d) a cereal; or

(e) a confectionary; or

(f) a flavour or beverage emulsion; or

(g) a fruit filling; or

(h) a gravy, soup, sauce or food thickener; or

(i) a frozen, chilled or ambient stable gravy, soup, sauce or food thickener; or

(j) a pasteurized, retorted or UHT treated gravy, soup, sauce or food thickener; or

(k) a meal or meal component, e.g., a vegetarian meal component; or

(l) a meat product (e.g., a comminuted meat product, sausage, burger, grill steak, canned meat, meat pie, fish, meat spread and paste); or

(m) a petfood; or

(n) a pharmaceutical or neutraceutical (e.g., a healthfood); or

(o) a potato product; or

(p) a dairy product (e.g., an ice-cream, dessert, milk drink, milk shake, yoghurt, cheese, cheese spread or dip); or

(q) a dressing (e.g., a salad or low fat dressing); or

(r) a snack or cracker; or

(s) a spread (e.g., a savoury or sweet spread); or

(t) a pasta product (e.g., a noodle); or

(u) a fat-filled powder (e.g., a non-dairy creamer); or

(v) a quiche or flan; or

(w) a textured vegetable protein (e.g., a textured vegetable product); or

(x) a vegetarian grill-steak; or

(y) a pate (e.g., a vegetarian pate) or spread; or

(z) a vegetable or meat extract; or

(a') a low fat spread, cheese or cream mimetic; or

(b') animal or fish feeds (e.g., baits and lures).

37. A cosmetic comprising the emulsion or gel of any one of Claims 23 to 34, the cosmetic for example being:

(a) a cream (e.g., face cream); or

(b) a lipstick; or

(c) a deodorant carrier; or

(d) a lotion; or

(e) a hair gel; or

(f) a soap (e.g., liquid soap); or

(g) a skin care product (e.g., sun lotion).

38. A crop protection composition, agrochemical, pesticide, oil reclamation composition, mould, casting, paint, ink, lubricant, encapsulation system, moisture barrier comprising the protein composition, emulsion or gel of any one of Claims 21 to 34.

39. A process for the production of an emulsion as defined in any one of Claims 23 to 32 comprising the steps of:

(a) providing a lupin protein composition (e.g., by a process as defined in any one of Claims 1, 3 and claims dependent thereon);

(b) mixing the protein composition of step (a) with oil and water;

(c) emulsifying the mixture of step (b).

40. A process for the production of a gel as defined in Claim 33 comprising the steps of:

(a) providing a lupin protein composition;

(b) mixing the protein composition of step (a) with water;

(c) gelling the mixture of step (b), for example by heating.

41. The process of Claim 39 or 40 which is carried out *in situ* within a foodstuff, drink or animal feed during processing thereof (e.g., during mixing, homogenization, cooking or heating thereof).

42. The process of Claim 39 or Claim 40 wherein step (a) comprises the step of isoelectrically precipitating lupin protein.

43. The process of Claim 42 wherein step (a) further comprises the preliminary steps of:

(a) providing lupin seeds;

(b) debittering the lupin seeds.

44. The process of any one of Claims 41 to 43 further comprising restructuring the isoelectrically precipitated lupin protein by the post-isoelectric precipitation/washing steps of, in the absence of substantial shearing forces:

(a) holding an aqueous slurry of the isolectrically-prepared protein at an alkaline pH and a treatment temperature of 75-95°C for 1-120 minutes (e.g., 1 to 60 min);

(b) neutralizing the treated slurry e.g. to a pH of 6.8 to 7.0; and optionally

(c) evaporating the neutralized slurry and drying it (for example by spray drying).

45. The process of Claim 44 wherein the aqueous slurry has a solids content of 12-25% by weight, e.g., 12-17% by weight.

46. The process of Claim 44 or Claim 45 wherein the alkaline pH is 7.5-9.0 or 9.5, e.g., 7.5-8.5.

47. The process of any one of Claims 44-46 wherein the treatment temperature is 80-95°C, for example about 85°C.

48. The process of any one of Claims 44-47 wherein the solubility of the lupin protein is decreased by the treatment.

49. An emulsion or foodstuff, drink or feed obtainable by the process of any one of Claims 39-48.

50. A method for increasing the oil binding functionality of a lupin protein composition comprising the steps of:

(a) holding an aqueous slurry of a lupin protein composition at an alkaline pH at an elevated temperature; and

(b) neutralising the treated slurry to produce a composition comprising restructured lupin protein (and optionally further comprising the step of evaporating and drying (e.g., by spray drying) the neutralised slurry).

51. A method for increasing the oil binding functionality of a lupin protein composition comprising the steps of:

(a) providing a composition comprising at least partially denatured lupin protein;

(b) holding an aqueous slurry of the at least partially denatured lupin protein composition at an alkaline pH at an elevated temperature; and

(c) neutralising the treated slurry to produce a composition comprising restructured lupin protein (and optionally further comprising the step of evaporating and drying (e.g., by spray drying) the neutralised slurry).

INTERNATIONAL SEARCH REPORT

Ir ational Application No
PCT/ 99/07106

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A23J3/14 A61K7/06 A61K7/48 A23G9/02 A23K1/16
A23L2/66

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A23J A61K A23G A23K A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category ³	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 522 800 A (DALGETY PLC) 13 January 1993 cited in the application	1,2,4,5, 7-50
Y	see the whole document ---	3,6,51
Y	US 5 210 184 A (CHAJUSS DANIEL) 11 May 1993 see the whole document ---	3,6,51
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

³ Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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Date of the actual completion of the international search	Date of mailing of the international search report
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INTERNATIONAL SEARCH REPORT

In [redacted]tional Application No
PCT[redacted] 99/07106

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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